Neonatal lead exposure impairs development of rodent barrel field cortex

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Childhood exposure to low-level lead can permanently reduce intelligence, but the neurobiologic mechanism for this effect is unknown. We examined the impact of lead exposure on the development of cortical columns, using the rodent barrel field as a model. In all areas of mammalian neocortex, cortical columns constitute a fundamental structural unit subserving information processing. Barrel field cortex contains columnar processing units with distinct clusters of layer IV neurons that receive sensory input from individual whiskers. In this study, rat pups were exposed to 0, 0.2, 1, 1.5, or 2 g/liter lead acetate in their dam's drinking water from birth through postnatal day 10. This treatment, which coincides with the development of segregated columns in the barrel field, produced blood lead concentrations from 1 to 31 μg/dl. On postnatal day 10, the area of the barrel field and of individual barrels was measured. A dose-related reduction in barrel field area was observed (Pearson correlation = −0.740; $P < 0.001$); mean barrel field area in the highest exposure group was decreased 12% versus controls. Individual barrels in the physiologically more active caudoventral group were affected preferentially. Total cortical area measured in the same sections was not altered significantly by lead exposure. These data support the hypothesis that lead exposure may impair the development of columnar processing units in immature neocortex. We demonstrate that low levels of blood lead, in the range seen in many impoverished inner-city children, cause structural alterations in a neocortical somatosensory map.

Childhood lead poisoning persists as a major public health problem throughout the world, despite efforts to reduce lead hazards in the environment. Although the mean blood lead level in the U.S. has decreased dramatically over the past 20 years, lead poisoning remains common among poor urban children (1). For example, of the 68,800 children tested in Baltimore from 1996 to 1998, more than 25% had elevated blood lead levels (≥10 μg/dl), and approximately 5% suffered lead poisoning (≥20 μg/dl; ref. 2).

Lead is especially damaging to the child's brain, causing a decline in intelligence that is correlated to blood lead level at age 2 (3–5). Deficits in learning have also been observed in rodents exposed to lead (6); these learning deficits have been associated with changes in glutamate receptor binding sites (7). Rats exposed to low levels of lead during the first postnatal month have deficits in memory and long-term potentiation that persist in the adult (8, 9). We hypothesize that the persistent detrimental effects of neonatal lead exposure may be due, in part, to deficits in neocortical development. The present study examines the effect of neonatal lead exposure on the morphology of cortical columns, using the rodent barrel field model.

The barrel field of somatosensory cortex receives its input from large whiskers, or vibrissae, on the rodent whisker pad. The whisker pad is arranged in five rows; each vibrissal follicle is innervated by sensory fibers that project in a highly ordered manner via the brainstem and thalamus to primary somatosensory cortex. The map of the whisker pad is faithfully replicated in layer IV of primary somatosensory cortex (Fig. 1), where small neurons aggregate into special structures termed "barrels" (10, 11). Cells within each barrel are functionally activated by one whisker (12, 13). The array of larger barrels representing the long whiskers on the snout is called the posteriomedial barrel subfield (which excludes the smaller barrels corresponding to short whiskers on the nose); this subfield is referred to in this report as the barrel field.

The topographic maps in the whisker to barrel pathway unfold sequentially, beginning in the periphery. Thalamocortical axons arrive in the developing cortex during embryonic life (14) but do not form a topographic pattern of clustered terminals in the barrel field until the first postnatal days (15–17). The characteristic aggregates of cells that constitute the barrels begin to form at P3 (18, 19). Subsequently, oriented growth and regression of dendrites sharpen the boundaries of each barrel (20).

The barrel of rodent somatosensory cortex is a special case of the cortical column. The column is the basic functional unit of neocortex and is comprised of a tangentially restricted area that extends through all six cortical layers (21, 22). Connectivity is enhanced within the column and restricted outside the column. Replication of this basic columnar unit within cortical areas permits the representation of relationships such as topography across sets of columns in an orderly fashion. The striking clusters of neurons that form barrels in layer IV of the rodent somatosensory cortex are unique. However, the segregation of thalamocortical afferents to small groups of layer IV neurons within a column is a fundamental characteristic of all types of sensory cortex. The rodent barrel field provides a useful model of cortical development, because the segregation of afferents is accompanied by clustering of the layer IV neurons into barrels that can be evaluated morphometrically.

Materials and Methods

Lead Exposure. In the first study, six pregnant female Long–Evans rats (Charles River Breeding Laboratories) were obtained at gestational day 14–16. Beginning on the day of parturition (P1), the dams received 0 ($n = 2$) or 1.5 ($n = 3$) g/liter lead acetate in their drinking water. In the second study, 12 timed-pregnant female Sprague–Dawley rats (Zivic Labs, Zelienople, PA) were obtained at gestational day 14 or 16. Beginning on P1, the dams received 0, 0.2, 1, or 2 g/liter lead acetate in their drinking water (three dams per exposure level). All dams were housed individually with a 12-h light/12-h dark schedule; all litters were culled to eight pups on P1 to prevent differences in body weight that may be caused by lead exposure. ANOVA revealed no significant effect of lead exposure on body weight on P10.

Tissue Preparation. On P10, three rats per litter were deeply anesthetized (600 mg/kg chloral hydrate i.p.) and perfused

Abbreviations: CO, cytochrome oxidase; NMDA, *N*-methyl-D-aspartate; P*n*, postnatal day *n*.

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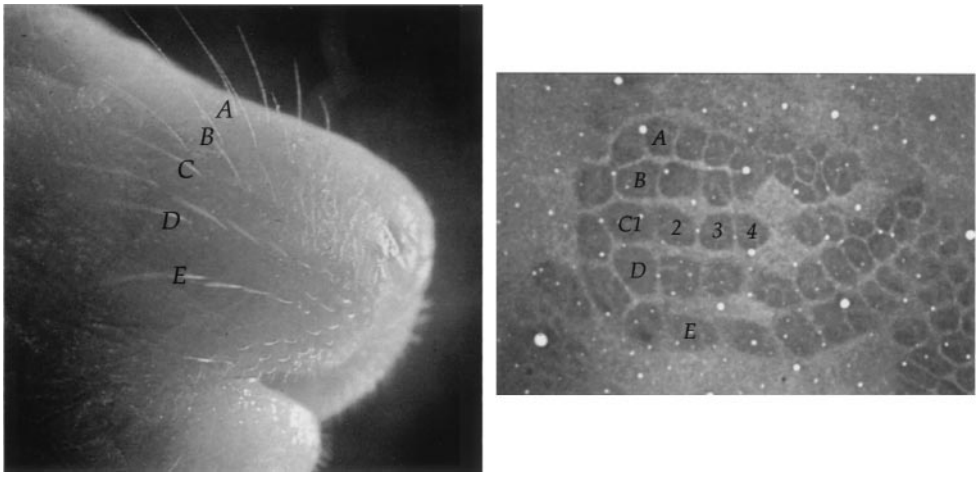

Fig. 1. Topography of whisker pad and cortical barrel field. The individual whiskers on the rodent whisker pad are arranged in five rows [*Left*; postnatal day 1 (P1)]. This topographic arrangement is replicated in layer IV of somatosensory cortex, where thalamocortical afferents and their target neurons aggregate into clusters termed barrels (*Right*; CO histochemistry; P10).

transcardially with 70 ml of PBS followed by 120 ml of 4% (wt/vol) paraformaldehyde in 0.15 M phosphate buffer (pH 7.4). Brains were removed, immersed for 5 h in the same fixative, and placed overnight in 15% and then 30% (wt/vol) sucrose in PBS. The brainstem was removed, and the cortex with underlying hippocampus was dissected away from the thalamus and striatum. These cortical blocks were flattened between two foil-wrapped glass slides that were held 1 mm apart by two squares of dental wax and were then frozen in powdered dry ice. The remaining five rats per litter were deeply anesthetized, and blood samples were obtained by cardiac puncture for determination of blood lead content. (Brains from these rats were removed and quickly frozen in powdered dry ice for use in other studies.)

In the first study, the barrel field was delineated in two sets of sections that were processed for immunohistochemical detection of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor subunits (GluR2,3; sections 4, 7, 10, and 13) or class II metabotropic glutamate receptor subunits (mGluR2,3; sections 6, 9, 12, and 15). In the second study, the barrel field was delineated with cytochrome oxidase (CO) histochemistry in serial sections throughout the depth of cortex.

Immunohistochemical Detection of Glutamate Receptors. Serial 50-μm frozen sections of the flattened cerebral cortex were cut on a sliding microtome and stored in an antifreeze solution at -20°C until processed. Free-floating sections were rinsed three times in PBS for 10 min at room temperature and then preincubated for 1 h in PBS containing 0.2% (vol/vol) Triton X-100, 4% (vol/vol) normal goat serum, and 0.2% (wt/vol) gelatin. Sections were then incubated for 72 h at 4°C in the same solution containing rabbit anti-GluR2,3 (1:500, generously provided by Lee J. Martin, Johns Hopkins Univ. School of Medicine, Baltimore) or mGluR2,3 (1:1,000, Chemicon). These affinity-purified polyclonal antibodies are directed against C-terminal receptor subunit peptides (23, 24). Control sections were incubated in the same dilution of normal rabbit serum. The antigen–antibody complex was visualized with the avidin–biotin peroxidase complex method (25) by using an ABC ELITE kit (Vector Laboratories).

CO Histochemistry. Serial 50-μm frozen sections of the flattened cerebral cortex were cut on a sliding microtome, collected in PBS, stored overnight at 4°C, and incubated in 0.1 M phosphate buffer (pH 7.4) containing 130 mM sucrose, 1.54 mM 3,3′-diaminobenzidine tetrahydrochloride, and 90 μM cytochrome *c* at 37°C, in the dark, for 7–9 h. Histochemical staining was evaluated under a dissecting microscope after 4 h and every 30 min thereafter and was terminated by rinsing the sections four times for 5 min in PBS. Sections were then mounted on gelatin-coated slides, dehydrated, cleared, and coverslipped.

Quantitative Morphometric Analysis. A computerized image analysis system (MCID, Imaging Research, St. Catherine's, ON, Canada) was used to measure the area of the barrel field in each animal. The barrel field was observed in approximately five serial sections of layer IV in each animal, cut parallel to the cortical surface. Digitized images of the three middle sections through the barrel field were aligned by using blood vessels as fiducial points. The boundary of the barrel field was delineated in the aligned images, and its area was measured. Areas were measured in three rats per litter, and the litter mean value was used for statistical comparisons. To combine the data from the two experiments, which used different strains of rats and different methods for delineating the barrel field, barrel field areas were normalized by expressing each as a percentage of the mean of the controls for that experiment. All statistical comparisons were made by using SPSS for WINDOWS with each litter mean value treated as an *n* of 1. ANOVA and regression analyses were used to evaluate the overall effect of lead exposure on the morphology of the barrel field; Dunnett's pairwise multiple comparisons post hoc test was used to compare the area of the barrel field in each lead-exposed group with that of controls.

In the second experiment, because every serial section through the barrel field was processed for CO histochemistry, measurement of individual barrels was possible. The area of individual barrels in columns 1–4 of rows A–E was measured in the single section that contained the clearest, largest image of each barrel. Row and column areas were calculated by summing the areas of the individual barrels measured in each row or column. Areas were measured in three animals per litter, and the litter mean value was treated as an *n* of 1 for statistical comparisons. Regression analyses were used to evaluate the effect of increasing lead exposure on the area of barrels, rows, and columns.

To obtain an estimate of relative cortical volume, the area of cerebral cortex was measured in each animal in one of the sections of flattened cortex used to measure the barrel field. The section with the most complete image of the barrel field was selected (usually section 7). Because the cortical volume was flattened to a comparable thickness in all blocks, the area of

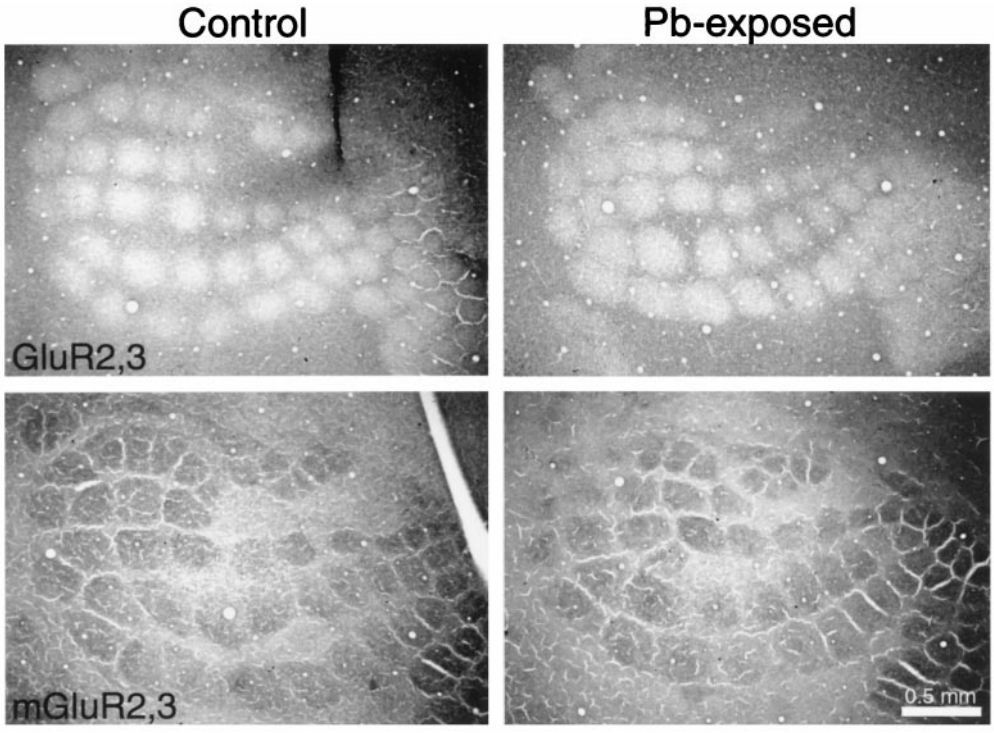

Fig. 2. Expression of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (GluR2,3) and metabotropic (mGluR2,3) glutamate receptor subunits on P10 in the barrel field of control and lead-exposed rats. Lead exposure does not alter the somatotopic pattern in the barrel field.

cortex measured in the resulting sections provides an estimate of relative cortical volume. The mean area of cortex for each litter was calculated from the three animals examined per litter and used for statistical comparisons.

Blood Lead Content. Blood samples from two to five rats per litter were analyzed for lead content by graphite furnace atomic absorption spectrophotometry in the Trace Metals Analysis Laboratory at Kennedy Krieger Institute (26). The mean blood lead levels for each litter and for each treatment group were calculated. Bivariate regression analysis was used to examine the relationship between lead exposure (percentage of lead acetate in dam's water) and mean blood lead level for each litter.

Results

Blood lead content was measured in littermates of the animals used for cortical barrel field measurements. In the initial experiment, Long–Evans rats were exposed to 0 or 1.5 g/liter lead acetate via the dam's drinking water from birth to P10. The mean blood lead observed in lead-exposed rats was 31 ± 2.2 (SEM) μg/dl; blood lead in controls was <1 μg/dl. In the second study, Sprague–Dawley rats were exposed to 0, 0.2, 1, or 2 g/liter lead acetate in the dam's drinking water; resulting mean blood lead levels were 1.4 ± 0.2, 6.8 ± 0.3, 19.1 ± 1.3, and 19.4 ± 0.9 μg/dl, respectively. Blood lead concentration increased with increasing lead content in the dam's drinking water (Pearson correlation = 0.879; $P < 0.001$).

In the first study, immunohistochemical detection of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and metabotropic glutamate receptors clearly delineated the barrel field (Fig. 2). There were no apparent differences in the overall appearance of the barrel field topographic pattern nor were there qualitative differences in the expression of the glutamate receptor subunits examined. In the second experiment, the barrel field was delineated by CO histochemistry (Fig. 1), and, as in the initial experiment, the overall pattern of barrel rows and columns was comparable.

In rats exposed to low levels of lead from birth to P10, the total area of the barrel field in primary somatosensory cortex, normalized as percentage of control mean for each experiment, decreased with increasing lead exposure (Fig. 3*a*; Pearson correlation = −0.740; $P < 0.001$; two tailed) and with increasing blood lead levels (Fig. 3*b*; Pearson correlation = −0.570; $P < 0.05$). ANOVA indicated a significant difference among groups exposed to different concentrations of lead ($P < 0.05$), and Dunnett's post hoc test indicated that barrel field area was reduced in rats exposed to 1.5 or 2 g/liter, compared with that of controls (10% smaller, $P < 0.05$; 12% smaller, $P < 0.01$, respectively).

The total area of cortex in rats exposed to 0, 0.2, 1, 1.5, or 2 g/liter lead acetate was 100 ± 1.2 (SEM), 97.8 ± 2.3, 99.0 ± 1.0, 88.0 ± 1.6, or 93.4 ± 8.1% of the mean in controls for each experiment. ANOVA detected no significant difference among the exposure groups in percentage of control mean cortical area, measured in the same sections used for the barrel field measurements. Regression analysis detected no significant relationship between lead exposure and total area of cortex.

To determine whether the area of particular rows, columns, or individual barrels was decreased by lead exposure, individual barrel areas were measured in columns 1–4 of rows A–E. These individual barrel areas were summed to obtain the areas of rows and columns. The effect of lead exposure on the area of each row is shown in Fig. 4. Bivariate regression analyses indicated that the area of rows B, C, and D decreased significantly with increasing neonatal lead exposure ($P < 0.05$ for rows B and C; $P < 0.01$ for row D). In these rows, mean row area was reduced 11–13% in the highest exposure group compared with that in controls. There was no significant correlation between the area of row A and lead exposure, and in row E, there was a trend toward smaller area with increasing lead exposure ($P = 0.09$).

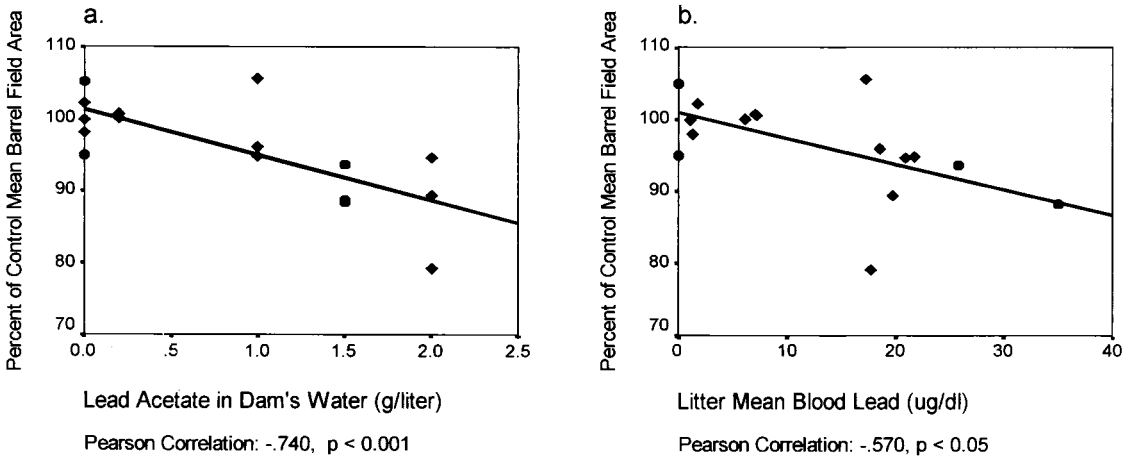

Fig. 3. (*a*) Reduction in barrel field area with increasing neonatal lead exposure. (*b*) Reduction in barrel field area with increasing blood lead concentration. Each point represents the mean of three rats examined per litter, normalized as percentage of the control mean for that experiment. ●, experiment 1; ◆, experiment 2.

The effect of neonatal lead exposure on the area of columns 1–4 is shown in Fig. 5. The area of column 1 decreased significantly with increasing neonatal lead exposure ($P < 0.01$). The mean area of column 1 was reduced 16% in the highest exposure group compared with that of controls. A trend toward smaller area with increasing exposure was observed in column 2 ($P = 0.08$). Columns 3 and 4 were not reduced significantly by lead exposure.

The area of some individual barrels decreased significantly with increasing lead exposure; the results of regression analyses for each of the barrels examined are summarized in Fig. 5*e*. The areas of all four barrels examined in row D were

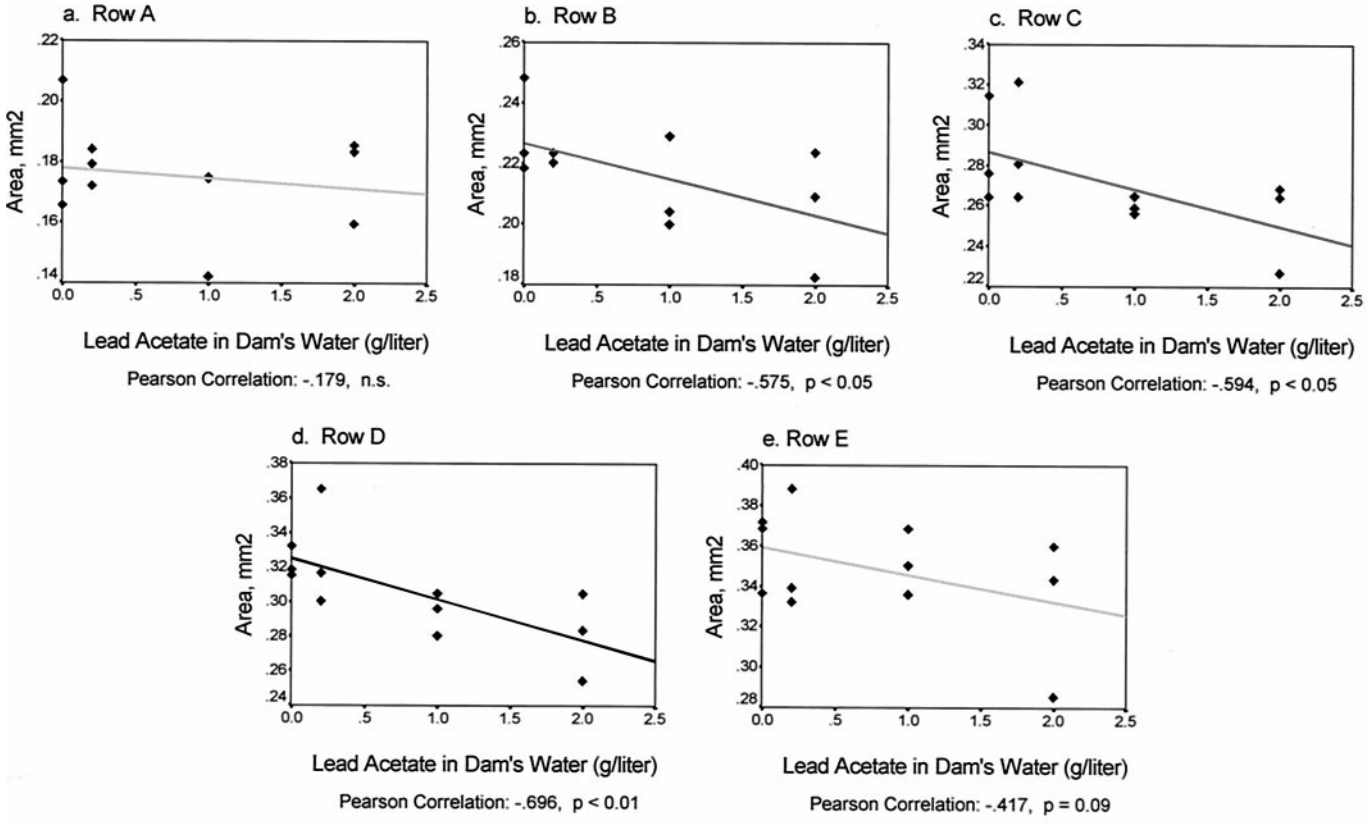

Fig. 4. (*a–e*) Negative correlation between lead exposure and the area of barrel field rows. The area of individual barrels in columns 1–4 of rows A–E was measured; individual barrel areas were summed within rows, and the litter mean value for each row was plotted against lead exposure level. Significant negative correlations were observed in rows B, C, and D.

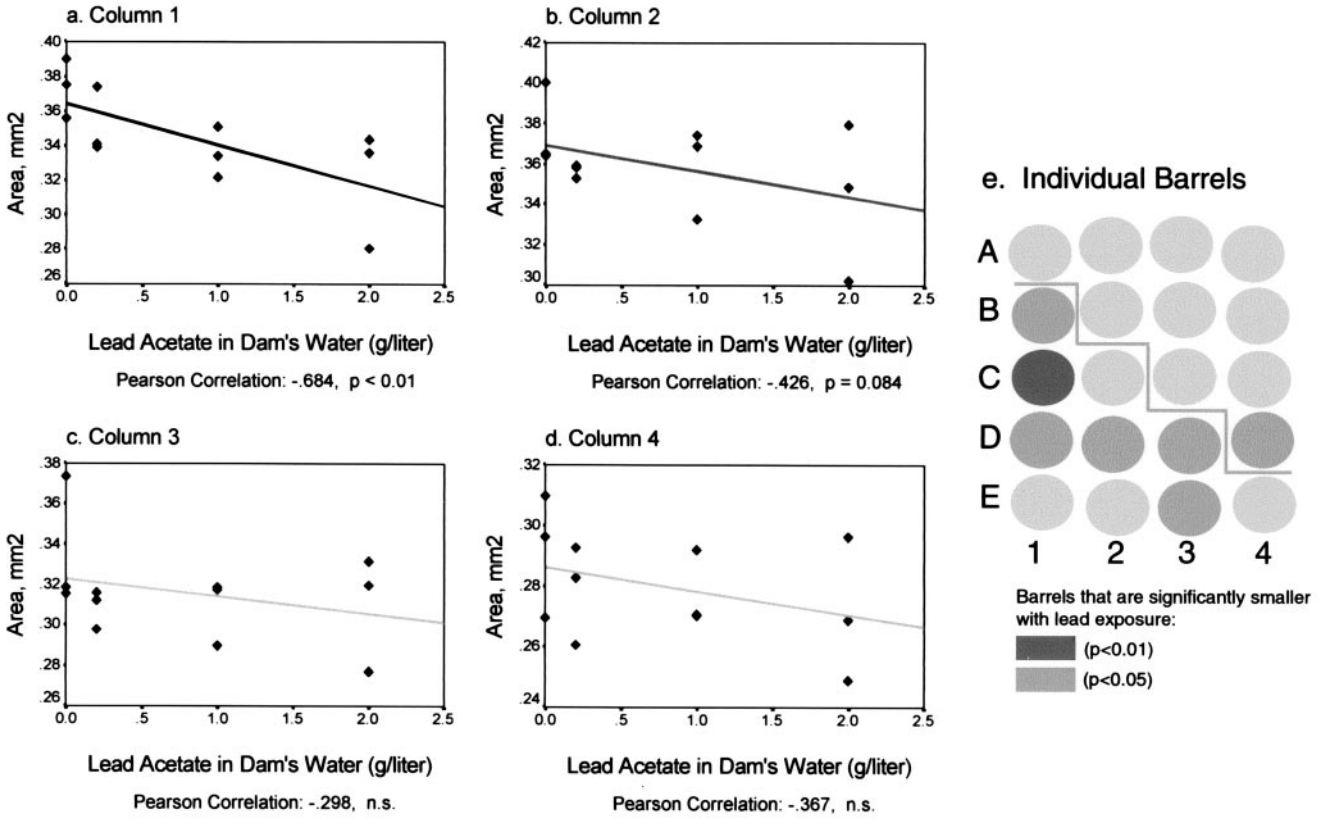

Fig. 5. (*a–d*) Negative correlation between lead exposure and the area of barrel field columns. The area of individual barrels in columns 1–4 of rows A–E was measured; individual barrel areas were summed within columns, and the litter mean value for each column was plotted against lead exposure level. A significant negative correlation was observed in column 1. (*e*) Summary diagram showing the spatial distribution of barrels that exhibit a significant negative correlation with lead exposure (regressions not shown). The gray line separates the barrel field into parts representing rostrodorsal and caudoventral whiskers. A significant negative correlation between barrel area and lead exposure was observed more frequently in barrels representing caudoventral whiskers.

reduced by lead exposure, and three of five barrels in column 1 were reduced by lead exposure, as was barrel E3. Thus, the reduction in total barrel field area reflects reductions in the area of individual barrels. Barrels that represent caudoventral whiskers were more likely to be affected by lead exposure (6 of 10 were affected) than those that represent rostrodorsal whiskers (1 of 10 were affected).

Discussion

In the present study, rats were exposed to lead during the period in which thalamocortical axon terminals form a topographic pattern in the barrel field and their target neurons cluster into barrels. The general appearance of the barrel field, with its orderly rows and columns of barrels, was comparable in control and lead-exposed animals, indicating that the mechanisms involved in establishing this topographic pattern are not markedly altered by low-level lead exposure. However, lead exposure produced a dose-related reduction in barrel field area and in the area of some individual barrels. Thus, lead exposure during the period in which barrel field topography is established restricts the size of cortical columns in this part of neocortex. The magnitude of this effect is modest, with 10–12% reductions in barrel field area observed at blood lead levels of 20–30 μg/dl.

Thalamocortical afferents in the barrel field contain the neurotransmitter glutamate, and the expression of glutamate receptors in the barrel field changes markedly over the first few weeks of postnatal development (27–29), suggesting that glutamatergic neurotransmission may be important in barrel field development. Exposure to low levels of lead modifies glutamatergic neurotransmission in several ways. Lead alters *N*-methyl-D-aspartate (NMDA)-type glutamate receptor activity (30, 31). Low concentrations of lead enhance glutamate and NMDA-induced currents *in vitro* (32). The magnitude and direction of this effect depend on the subunit composition of the NMDA receptor, which is regulated developmentally and varies among brain regions. Thus, the interaction of lead with NMDA receptors *in vivo* is likely to vary, depending on the concentration and length of exposure, the age of the subject, and the brain region.

Lead exposure also alters the expression of NMDA receptors. Exposure to lead during gestation and postnatal life increases expression of NR1 glutamate receptor subunit mRNA and decreases expression of NR2A mRNA and protein in the hippocampus (33, 34). A lead-induced delay in postnatal expression of NR2A subunits would be expected to prolong Ca^{2+} currents through the NMDA receptor (35). Lead has been shown to increase intracellular Ca^{2+} levels (36) and directly increases protein kinase C activity (37), cellular mechanisms that are downstream from NMDA and metabotropic glutamate receptors. Taken together, the evidence suggests that the effect of lead on the postnatal rat cortex may be comparable to an increase in glutamate receptor activity.

How would a change in NMDA receptor activity affect axonal arborization and barrel size? Local administration of MK-801 or infraorbital nerve section increases the size of barrels and the width of thalamocortical terminal arbors in the barrel field (38, 39). A reduction in axonal arborization and synaptic contact is induced in frog optic tectum chronically exposed to NMDA (40), an effect that resembles the effect of lead on axonal arbors in that

system (41). Exposure to high levels of lead has been shown to decrease dendritic branching and synaptic density in rat cerebral cortex (42). These studies suggest that lead-induced changes in glutamatergic neurotransmission or mechanisms downstream from glutamate receptors may reduce axonal and dendritic arborization and decrease barrel area.

Although many individual barrels representing caudoventral whiskers were affected by lead exposure, few representing rostrodorsal whiskers were affected. Barrels representing caudoventral whiskers have been shown to have a higher activity level (43), suggesting that more active barrels are more vulnerable to the effects of lead. This observation may relate to the special vulnerability of young children to lead, because positron emission tomography measurement of glucose utilization indicates a peak of cerebral metabolism at this age (44).

Exposure to high levels of lead from P1 to P25 has been shown to decrease brain weight by 13.2% and cortical thickness by 13.9% (42). The much lower levels of lead exposure used in the present study had no detectable effect on the total area of the flattened cortex at P10. Thus, there was not a global effect in all parts of cerebral cortex comparable with that observed in the barrel field. The period of exposure in the present study encompasses the critical period of enhanced morphologic plasticity for the barrel field (45). Other cortical regions, such as visual cortex, may be less vulnerable to lead exposure during this window because they have different critical periods for experience-dependent plasticity (46, 47).

Cortical columns in the barrel field of the developing rat exhibit a dose-related reduction in size after exposure to low levels of lead. These findings are consistent with a model in which lead alters glutamatergic neurotransmission, which in turn limits arborization of thalamocortical axons and reduces barrel field area. We have shown that low levels of lead exposure, in the range seen in many impoverished inner-city children, cause structural alterations in a neocortical somatosensory map.

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